Review article

Retinal functional imager (RFI): Non-invasive functional imaging of the retina

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Abstract

Retinal functional imager (RFI) is a unique non-invasive functional imaging system with novel capabilities for visualizing the retina. The objective of this review was to show the utility of non-invasive functional imaging in various disorders. Electronic literature search was carried out using the websites www.pubmed.gov and www.google.com. The search words were retinal functional imager and non-invasive retinal imaging used in combination. The articles published or translated into English were studied. The RFI directly measures hemodynamic parameters such as retinal blood-flow velocity, oximetric state, metabolic responses to photic activation and generates capillary perfusion maps (CPM) that provides retinal vasculature detail similar to flourescein angiography. All of these parameters stand in a direct relationship to the function and therefore the health of the retina, and are known to be degraded in the course of retinal diseases. Detecting changes in retinal function aid early diagnosis and treatment as functional changes often precede structural changes in many retinal disorders.

Keywords: Retinal functional imager, non-invasive retinal imaging

Introduction

Newer retinal imaging technologies help us in understanding the pathogenesis of many retinal pathologies such as diabetic retinopathy, age related macular degeneration, glaucoma and uveitis. Early detection and monitoring of these retinal diseases can prevent the onset of progressive vision loss and blindness. Imaging techniques can also provide a better understanding of the pathogenesis, aiding in the development of new treatment options. Most of the available newer retinal imaging tools addresses structural rather than functional changes. Retinal Functional Imaging (RFI) offers a noninvasive diagnostic approach to retinal function assessment and provides easy, direct, qualitative, and quantitative imaging of parameters including: (1) Blood-flow velocity (2) Capillary perfusion maps and microvasculature enhancement (3) Blood Oximetry (4) Metabolic intrinsic state/function (Nelson DA et al 2005).

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The objective of this review was to show the utility of non-invasive functional imaging in various disorders. Electronic literature search was carried out using the websites www.pubmed.gov and www.google.com. The search words were retinal functional imager and non-invasive retinal imaging used in combination. The articles published or translated into English were studied.

The RFI's imaging approach is similar to the one first used in the brain. Functional optical imaging is based on slow changes in intrinsic properties of active brain tissue is optimized for the architecture of the neocortex. This facilitated in vivo visualization of active cortical regions with

the unprecedented spatial resolution of 50 micron or less. It reveals activity-dependent changes in light reflectance, recorded using a camera with high spatial and temporal resolution. Functional signals are usually small, originating from activity-dependent metabolic, hemodynamic, and fast and slow light-scattering.

Adapting brain functional imaging techniques to the retina provides imaging of functional parameters directly related to the vitality and viability of the retina unlike most current powerful technological methods that only evaluate structural changes in the retina.

The RFI is capable of directly determining blood-flow velocity in the secondary and tertiary vessels of the microvasculature by tracking erythrocytes. Further analysis of the erythrocyte movement in the retina generates microvascular maps, capillary perfusion maps (CPMs) that complement the functional blood flow velocity measurements. The RFI also measures the variation in the reflected light with respect to the wavelength, assessing relative concentration of hemoglobin chromophores in both vessels and capillary background for information about intravascular oxygen content (oximetry maps) related to tissue vitality(Denninghoff KR et al 2002, Harris A et al 2003).

Principles and recording protocols

RFI system combines digital fundus imaging and functional optical imaging, with flexible parameter changes and standard data storage. Certain enhancements have been incorporated in the fundus camera to facilitate functional imaging.

1) ***Rapid sequential imaging***: A 60 Hz, 1024X1024 pixel digital imaging system uses a stroboscopic flash lamp system to take snapshots of the fundus (35 degree of the central retina) at rates high enough to reduce inter-frame retinal motion and follow erythrocytes moving at up to 20 mm/sec. This sensitivity range is generally most appropriate for secondary and tertiary vessels, providing high resolution, region-specific flow information about the microvasculature.

2) ***Rapid delivery of illumination of sufficient intensity to permit low-noise imaging***: In each series eight consecutive flashes with an interflash interval of less than 20 ms is delivered to a patient, generating eight images under a second. Multiple series of eight frames are obtained from each in one session. The captured eight frame sequences can be presented as a movie.

3) ***Multi spectral imaging*** (Rapid changes in illumination wavelength): This is performed for oximetric measurement. A fast filter wheel integrated in the camera is capable of rapidly switching up to four Illumination wavelengths twice within 55 sec. This allows multiple wavelength image acquisition with little or no global eye movement.

4) ***Stimulus generator***: It uses visual patterned stimulus with a specified pattern, frequency and duration.

In a single session, multiple series, each with 8 frames are obtained (following pupil dilation) by methods familiar to operators of standard fundus cameras. High optical magnification (standard 20°or 35^0 settings on the fundus camera optics) is preferred for imaging modalities that rely on erythrocyte motion (including velocities and CPMs). Both narrow-and wide-angle settings are appropriate for oximetry. A subject may sit for 5–15 min per session, and image sequences and initial results can be quickly reviewed during the acquisition session, allowing reacquisition whenever needed. Three series with 8 high quality frames in each should be selected for blood flow measurements. The image should be of good quality with precise focus and exposure. Image quality is defined according to the percentage of vessels excluded. Any velocity results yielding a coefficient of variance (SD/ mean) greater than 45% are indicative of poor image quality. The raw data obtained in a single sequence resembles familiar red-free images, except that they have been taken only milliseconds apart, and at different wavelengths (e.g. for oximetry). Automatic algorithms perform sub-pixel re-registration of the images within a sequence and then apply differential

analysis to reveal the differences among them (Brown, LG 1992).This analysis reveals image differences due to the phenomena described above.

Retinal blood-flow

Under green illumination, hemoglobin within the erythrocytes in the bloodstream provides a natural, high-contrast chromophore (at wavelength between 530-590 nm) for tracking blood flow (Jensen PS and Glucksberg MR 1998). Cross-correlation matching of moving erythrocyte patterns within an image series gives a direct measure of velocity. The RFI measures velocities in second and tertiary branches of the main retinal vessels (both arteries and veins). These secondary and tertiary vessels as extensions of the central artery are responsible for the delivery of 20–30% of the total ocular blood flow to the inner retinal layers. The RFI is unique in that it allows studying flow velocity changes in a large number of arterioles and venules simultaneously. The red blood cells appear dark under green light and are arranged randomly along the length of the blood column. This creates a light and dark pattern along the vessel which is preserved even as it moves forward. The RFI's flow measurement technique looks for correlations between patterns imaged at different times in order to determine how far the pattern has advanced during the imaging interval.

The re-registration and differential processing of a series of images taken at 50-60 Hz produces a "flow movie", in which it is possible to follow the motion of individual clusters of red blood cells or even single red blood cell (Figure 1).

Flow velocity quantification is necessary for any objective study of the relationship of blood flow and disease. Manual, spot-by-spot measurement of distance moved per frame interval is the most direct method of quantifying flow velocity. Manual method is too cumbersome for regular use. Automated flow velocity quantification is achieved by using a path-constrained cross-correlation technique (Figure2A & 2B). Path templates (essentially tracings over the blood vessels chosen for analysis) are generated from combining user supervision with automatic detection. During quantification, flow is assumed to be constrained to follow the paths thus defined. Cross-correlation analysis is used to determine the relative offset of path segments in sequential images, which contain approximately the same pattern of moving blood cell. The size of this offset, suitably calibrated and multiplied by the frame rate, gives blood-flow velocity (Nelson DA et al, 2005).

A negative value indicates blood flow away from the heart, whereas a positive value indicates blood flow toward the heart. Landa et al, have showed in their study on RFI in normal individuals, the range of arterial blood flow velocity was between 3.7 and 5.8 mm/sec and the range of venous blood flow velocity was 3.0 and 4.5 mm/sec(Gennady Landa et al, 2009). Venous RBF, analyzed by the RFI, significantly correlated with the thickness of the central retina, measured by SLO-OCT .Venous blood velocity increased linearly with the increase in the central retinal thickness. The RFI may thus be a useful tool for evaluating changes in retinal thickening(Gennady Landa et al, 2009).

Finally, to facilitate the measurement of true flow (volume), or to permit cross-subject categorization of vessel flow velocity according to size, approximate vessel diameters can be obtained from the red-free images that comprise the primary data. The instrument's analysis software suite provides a simplified light absorption model that may be used to fit the vessel's measured profile, yielding approximate, but useful width measurements.

The RFl imaging system can clearly reveal the motion of individual clusters of red blood cells, providing a powerful tool for measurement of retinal blood dynamics. Currently, the RFI provides a velocity map; however, conversion of the velocity units to flow units has not yet been implemented. To accomplish this goal, the precise vessel diameter must be established, a task already accomplished by Blum et al (Blum M et al, 1999).

The RFl's direct visualization of retinal blood flow, without the injection of contrast agents, opens up many new diagnostic possibilities of abnormal retinal blood flow velocity, particularly in capillaries, arterioles and venules. RFI can be used to study different collateral vascular patterns in normal individuals as well as in various retinal diseases. Landa et al. in their study showed that four patterns of retinal collateral circulation (Looped pattern, Vertical pattern, H-shaped pattern and Cilioretinal-retinal collateral pattern) were noted in normal individuals and in patients with various retinal disorders(Landa G et al, 2010). RFI is also useful in many systemic diseases like diabetic retinopathy, hypertension and other retinal vascular disorders.

Visualization of invisible or obscure vessels

The "flickering" of pixels in a flow movie provides a source of contrast that distinguishes pixels that image in-focus blood vessels from those that do not. Plotting pixel value as a function of the standard deviation of each pixel over time thus produces a high resolution vascular map, calculated based on motion contrast, rather than total reflectance contrast .This technique reveals vessels which are indistinct or invisible in even a very sharp red-free image (Figure 3).

In RFI maps, since the movement of red cell clusters is more distinct and less motion blurring occurs, the clarity of smallest vessels is equivalent to that of large vessels. The resulting map non-invasively documents fine details of vascular anatomy that may otherwise be obscured or invisible.

Capillary Perfusion Map

Following image registration, pixel value distribution parameters are analyzed to locate blood motion, tracing microvasculature based on motion contrast rather than on total reflectance contrast. The RFI's noninvasive capillary perfusion map (CPM) shows vessels that are indistinct or invisible in even sharp red-free images. The recently improved algorithm for CPMs now generate images providing ,as much if not more details of the retinal microvasculature than corresponding fluorescein angiography (FA) images (Figure 4).

The RFI can assist in cases where adverse reactions to fluorescein injection have been observed (Kwan AS et al, 2006). The most significant improvements of CPM over FA are (1) they are noninvasive (2) more detailed (3) allow follow-up at any frequency and (4) show where red blood cells are moving at approximately normal speed. In the latter context, variations between normal capillaries and capillaries exhibiting reduced red blood cell velocity cannot be detected with FA. CPMs also provide better detail of microaneurysms in patients with DR, including non-leaking microaneurysms that are rarely seen with FA. At present, however, one cannot infer from CPM maps the regions of leakage that are readily detected with FA, and detection of leakage remains a future challenge for RFI noninvasive imaging.

Clinical Studies with the RFI shows special characteristics in diabetic patients with NPDR, The RFI reveals a significant velocity decrease compared to healthy controls (Delori FC 1998). In patients with early DM with no diabetic retinopathy, the RFI can detect an increased blood flow velocity compared to controls and CPM imaging showed an increase in foveal avascular zone in diabetic patients compared to healthy controls (Burgansky-Eliash Z et al, 2010). CPM imaging showed areas of capillary non-perfusion in patients with diabetic retinopathy and BRVO in correspondence with FA findings (Burgansky-Eliash Z et al, 2010).

Following ischemic area and changes in blood flow velocities are helpful in early diagnosis of conditions developing in diabetic eye as well as tailoring the treatment.

In AMD patients, RFI detected reduced blood-flow velocity in exudative AMD eyes compared with fellow dry AMD eyes. Average blood flow velocity in arteries and veins was significantly lower in AMD patients compared to controls.Drug effects on the retina have also been studied with the RFI (unpublished data).

Following intravitreal bevacizumab (Avastin) injections, a distinctive pattern of change in patient retinal blood-flow velocity was noted in responders versus non-responders. In retinal vascular occlusion patients neovascular loops are better imaged on RFI than on flourescein angiography (Figure 5).

In glaucoma patients RFI is useful in the evaluation of para papillary blood flow in microvasculature perfusing the optic disc as it relates to the vascular pathogenesis in different types of glaucoma (Figure 6).

Multispectral imaging for retinal oximetry

The difference between the absorption spectra of oxy- and deoxyhemoglobin can be used to determine the oxygenation of blood with spectroscopic methods.

Alterations in either oxygen supply or consumption might directly indicate the early ronset of retinal abnormalities (Stefansson E et al, 1983; Stefansson E et al, 1992; Tiedeman JS et al, 1998; Sebag J et al, 1989). Thus, it is important to have a tool for qualitative and quantitative assessment of oxygen utilization in the retina. In multi wavelength mode, RFI can perform spectroscopic decomposition to qualitatively assess the oximetric state of the retina (Figure 7).

The qualitative map has limited value compared to quantitative oximetry, but does have role in clinical conditions. However the optical complexity of the retina hampers the quantitative evaluation of oximetric maps (Burns SA et al 2003). Perfusion deficits and abnormalities appear as a region of color distinct from their surroundings. Poor perfusion areas appears blue, highly perfused area appears red (Figure 7).

In patients with diabetic retinopathy, regions appearing normal in fluorescein angiogram are patchy darker in the oximetric maps- suggesting ischemia. This underscores the significance of qualitative oximetric imaging as a supplement to angiography that can detect anoxia directly, rather than by inference from dye leaks. As with RFI CPMs, oximetry maps are also capable of providing a high-resolution detail of microaneurysms in patients with diabetic retinopathy, as they are not obscured by the leakage that occurs in FA.

Multispectral filter wheel allows choroidal vessels visualization using near infrared light and pigment density maps. The benefits of using the multi-spectral imaging oximetric include rapid non invasive imaging of the retina, providing results indicating oximetric state regions of ischemia and direct visualization of choroidal vessels without using a contrast agent. Current studies are examining changes in oximetry state in patients with diabetic retinopathy as a result of laser treatment.

Retina functional metabolic signal

RFI can detect functional signal originating from the reflectance changes in the retina upon visual stimulus .It reveals activity dependent changes in light reflectance, recorded using a digital camera with high spatial and temporal resolution. Such functional signals are usually small, originating from activity-dependent metabolic, hemodynamic, and fast and slow light-scattering Changes (Grinvald A et al: 1999: Hill DK and Keynes RD 1949: Cohen LB et al 1968; Cohen LB 1973; Frostig RD et al 1990; Malonek D and Grinvald A 1996).

The RFI is capable of imaging outside the absorption range of photoreceptors under near-infrared light (750–840 nm) and can be used to optically monitor retinal activity in response to a well-defined visual stimulus (562$\pm$20 nm) The difference between the post-stimulated and pre-stimulated images is used to determine the metabolic state of the retinal compartments(Fig 15&16).Retinal functional imager shows the metabolic state of the directly activated retina thus helps in imaging the functional state of the axonal arches, which are the activated axons of ganglion cells leading from the activated area to the optic nerve head .Also useful in imaging blood flow, blood volume & oximetric changes under photic activation.

Among the limitations of the RFI, is the fact that it is not a real-time device. It must be synchronized with patients' heartbeat using oximeter. Instrumental artifacts may arise from

imprecise focus and localization during image acquisition. Relatively clear ocular media is important for obtaining pictures with a good resolution, which often limits its use in patients with complicated ocular conditions. Depth of penetration is limited by the transparency of the retina, which varies with the illumination. Also the illumination wavelengths are restricted to the range of light at which hemoglobin absorbs well and which the eye returns in sufficient quantities to produce a good signal-to-noise level.

The RFI gives information on velocity only and not on flow volume because of resolution limitations. Future refinements of the technique and software enhancements should remedy some of these limitations. Acquisition of the highest quality images requires optimizing the focus of the captured image. This can be improved by an automated focusing system or enhancing depth of field by reducing the aperture size post-processing. These improvements would require faster, more sensitive components. Digital image focus enhancement is another possibility and also being pursued. The RFI's facility for acquiring multiple images with near simultaneity makes applications of these types of techniques a real possibility. Improvements in digital camera technology will also help to enhance RFI image quality.

In conclusion, the Retinal Functional Imager is a new clinically applicable method for estimation of the retinal blood flow velocity, perfusion and microvascular structure. It shows promise of being able to detect subtle circulation changes both in normal subjects and those with ocular disorders. Detection of functional parameter abnormalities may permit diagnosis of diseases and to evaluate disease progression before anatomic abnormalities become evident, allowing treatment intervention before irreversible retinal damage occurs. It also opens research and drug development opportunities respecting a wide range of retinal diseases, beyond the capabilities of structural imaging. Future modifications in RFI will help a long way in avoiding many invasive diagnostic retinal tools.

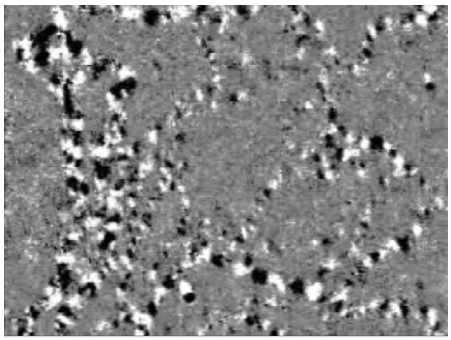

Figure 1: Differential images -Black spots are erythrocyte or erythrocyte Cluster. White spots or gaps, represent absence of erythrocytes. The direct nature of measurement allows simple inspection of a flow movie to quickly reveal gross abnormalities in blood flow.

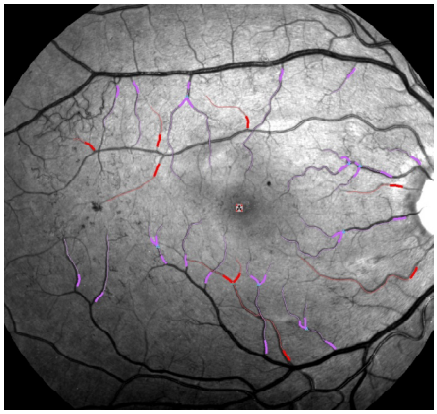

Figure 2A: Arteries (red) and veins (violet) that were manually selected for quantification.

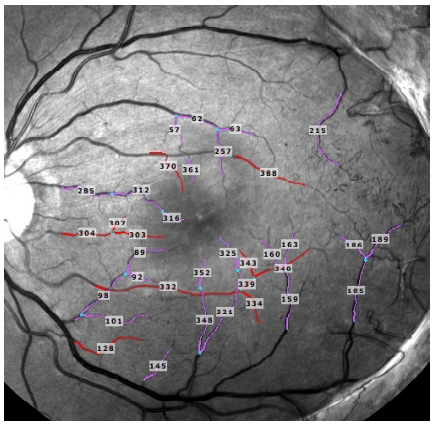

Figure 2B: Blood velocity map-Measured velocities in veins (positive values) and in arteries(negative values) are presented in millimeters per second value± SD.
(The average is based upon measurements from the three combined series)

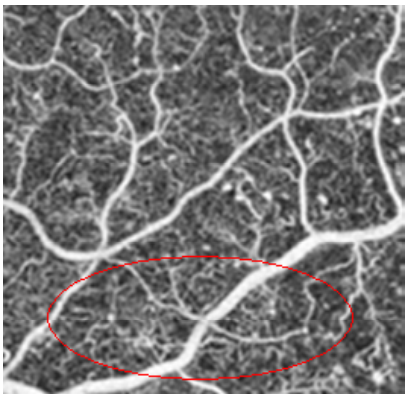

Figure 3: Shunt and anastomotic vessels better seen in Diabetic retinopathy on RFI

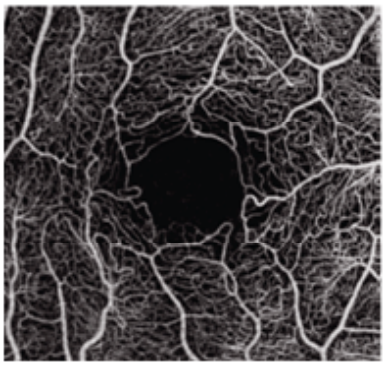

Figure 4: CapillaryPerfusion Maps are obtained without any contrast agent such as fluorescein. Instead the red blood cells serve as an intrinsic contrast agent. Their flow shows the positions of the veins, arteries and capillaries. Retinal microvasculature including surface capillaries are seen better than most of FA images

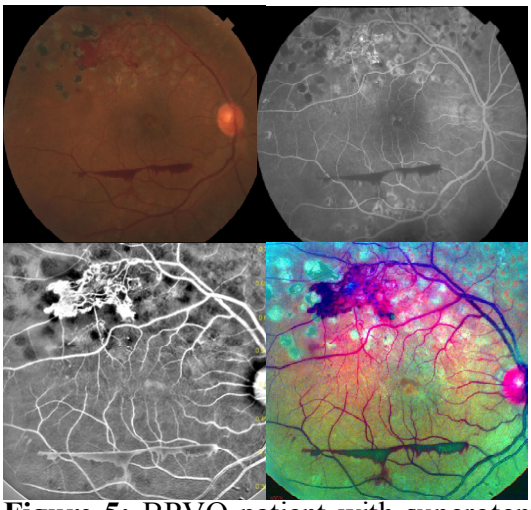

Figure 5: BRVO patient with superotemporal quadrant NVE better seen than on FA (colour FA and RFI

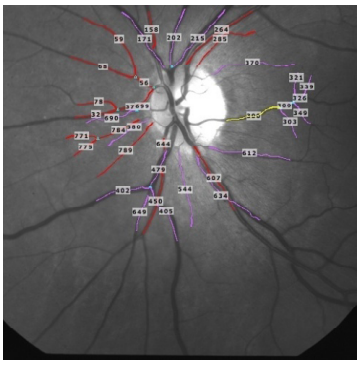

Figure 6: Para papillary blood flow measurement in a Glaucoma patient.

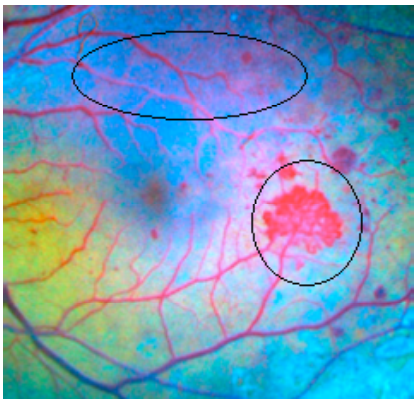

Figure 7: Sickle cell retinopathy with highly perfused sea-fan neovascular loops. Areas of poor perfusion are seen as blue on qualitative oximetry.

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